Electronic Supplementary Material (ESI) for Metallomics

Supporting Materials

Supplementary Method: Analytical method validation

A total of 22 single-element standard solutions of Li, B, Mg, Al, Ti, V, Cr, Fe, Cu, Ga, Se, Rb, Sr, Mo, Cs, Ba, Ce, Hg, Tl, Pb, Th, and U (ICP standard, Merck KGaA, Darmstadt, Germany) were used to make a mixed stock solution. The mixed solution was subsequently diluted to a series of concentration of 0.004, 0.01, 0.04, 0.1, 0.4, 1.0, 4.0, 10.0, 40.0, 100.0, 400, or 1000 μg/L using 2% HNO3, and the dynamic range and coefficient of determination (R2) were attained by the ChemStation software. The accuracy was measured by replicated analysis of 10 mixed solutions at a concentration of 0.4 μg/L. The pooled quality control (QC) samples were prepared by mixing aliquots of all of the study samples such that the pooled samples broadly represent the biological average of the whole sample set. The goal of using the pooled QC samples was to provide a set of data that can be used to assess overall reproducibility, recovery, and stability of the targeted elements in biological matrix. The reproducibility was evaluated by repeatedly injecting the same amount of digested serum samples at regular intervals (every ten study samples) throughout the analytical run. The recovery was calculated by comparing the actual amount of the 22 element standards added to the serum sample with the total amount of these elements extracted from the spiked mixture that was prepared by spiking the mixed solution of the 22 elements with the pooled serum sample. As microwave assisted digestion can process up to 40 samples per batch required in about 1 hr and running these samples through ICP-MS requires 3 hr, the total time of sample preparation and instrument analysis per batch (40 samples) was approximately 4 hr. In this case, short-term sample stability was evaluated by measuring the digested sample every 1 hour within 8 hours at a temperature- and humidity- controlled room (temperature : 20 ± 2 °C; humidity : 35-50%).

Table S-1. Sample gout questionnaire

Basic Information							
Name (printed)			**Date of birth (mm/yyyy)**		/		
Height (m)			**Weight (kg)**				
			Primary phone number				
Policy number			**Healthcare provider**				
Address			**Residence (province/ city)**				
Social History and Lifestyle							
Occupation		**Marital status**	married	divorced	separated	widowed	single
Use of alcohol	1. never	If your answer is "2" or "3", please specify the type(s) of alcohol beverage and average amount.					
	2. occasionally	beer	red wine	white wine	yellow wine		
	3. frequently	liquor	___ o.z. per	day	week	month	
	4. previously, but quit. When? (mm/yyyy)			/			
Use of tobacco	1. never	If your answer is "2" or "3", please specify the average quantity.					
	2. occasionally						
	3. frequently		___ package per	day	week	month	
	4. previously, but quit. When? (mm/yyyy)			/			
Diet	vegetables		___ times per	day	week	month	
	red meat (pork, beef etc.)		___ times per	day	week	month	
	white meat (chicken)		___ times per	day	week	month	
	eggs		___ times per	day	week	month	
	seafood (fresh and canned)		___ times per	day	week	month	
	organ meat (liver, kidneys, sweetbreads)		___ times per	day	week	month	
	legumes (dried beans, peas)		___ times per	day	week	month	
Indoor or outdoor exercise	1. never	If your answer is "2" or "3", please provide the details on frequency and duration of each time.					
	2. occasionally						
	3. frequently						

Questions								
1	Date of your first symptoms, episode, or attack of gout (mm/yyyy)			/				
2	Date first diagnosed (mm/yyyy)			/				
3	Please specify each part of your body being affected. (e.g., left elbow, right knee, finger, toes).							
4	Frequency of the attacks	hourly	daily	weekly	monthly	quarterly		
		yearly		one-off	other			
5	What was the average duration of each attack?							
6	When the attack occurs, does it affect your work or restrict your movements?	yes	If "yes", please provide details.					
		no						
7	Would you consider your severity of symptoms to be?	mild	moderate	severe				
8	The date of the last attack (mm/dd/yyyy)	/	/					
9	Please specify the complications (if any)							

10	Please list your current medications (prescriptions, non-prescription, vitamins, etc.. please attach extra sheet of paper if the space is insufficient):	medication	dose	frequency	reason for use

11	Family medical history	relative	disease	cause of death if deceased
		father		
		mother		
		sisters		
		brothers		
		spouse		

12	Please provide the date and result of your most recent serum uric acid level.	date (mm/dd/yyyy)	/ /	
		uric acid (μmol/L)		

I hereby declare that all of the above statements are true, complete and correctly recorded to the best of my knowledge. I also agree that the questions and answers above shall be used for research purpose.

Patient Signature: _____

Date: _____

Table S-2. Clinical Diagnosis of gout

Clinical diagnosis of gout (To be completed by clinicians only)				
Patient Name		**Policy number**		
1. Presence of characteristic urate crystals in the joint fluid		YES		NO
2. Presence of a tophus proven to contain urate crystals by chemical means or polarized light microscopy		YES		NO
3. Presence of six of the following clinical, laboratory, and radiographic phenomena:		YES		NO
3.1 More than one attack of acute arthritis		YES		NO
3.2 Development of maximal inflammation within 1 day		YES		NO
3.3 Attack of monarticular arthritis		YES		NO
3.4 Observation of joint redness		YES		NO
3.5 Pain or swelling in first metatarsophalangeal joint		YES		NO
3.6 Unilateral attack involving first metatarsophalangeal joint		YES		NO
3.7 Unilateral attack involving tarsal joint		YES		NO
3.8 Suspected tophus		YES		NO
3.9 Hyperuricemia		YES		NO
3.10 Asymmetric swelling within a joint on x-ray		YES		NO
3.11 Subcortical cysts without erosions on x-ray		YES		NO
3.12 Negative culture of joint fluid for microorganisms during attack of joint inflammation		YES		NO
GOUT		**YES**		**NO**
Metrics/criteria: The diagnosis of gout followed the 1977 American Rheumatism Association (ARA) criteria with modification. The clinicians from different hospitals were trained to use the same protocol in this study. The patients are considered gout if they meet 1 or 2 or any six symptoms of 3.				

Physician/Clinician Name (printed)		**Phone #**	
Facility Name			

Physician/Clinician Signature: _____

Date(mm/dd/yyyy): _____

Table S-3. Summary of validation on the analytical methods and procedures.

Element	Dynamic range (μg/L)	R^2 [a]	Accuracy (R.S.D., %)[b]	Precision (R.S.D., %)	Recovery (Mean±S.D.)	Stability (R.S.D., %)
Li	0.0—100.0	0.9996	2.61	4.54	115.61±2.37	4.12
B	0.0—100.0	0.9998	2.37	3.32	102.34±3.61	2.87
Mg	0.0—1000.0	0.9998	1.64	1.13	98.68±0.98	1.30
Al	0.0—1000.0	0.9999	3.25	2.98	94.56±6.75	2.95
Ti	0.0—100.0	1.0000	2.49	3.35	93.37±1.28	3.12
V	0.0—100.0	0.9998	1.57	3.31	113.16±3.82	2.46
Cr	0.0—100.0	0.9997	1.64	2.94	104.9±2.67	2.24
Fe	0.0—1000.0	0.9998	0.90	2.18	90.83±2.11	1.89
Cu	0.0—1000.0	1.0000	0.73	2.21	108.34±0.91	1.06
Ga	0.0—100.0	1.0000	3.07	7.36	106.26±2.48	5.57
Se	0.0—100.0	0.9997	1.40	2.32	96.8±3.16	3.13
Rb	0.0—100.0	0.9998	2.13	2.30	108.76±3.63	2.68
Sr	0.0—100.0	0.9999	1.70	1.61	104.88±2.77	1.39
Mo	0.0—100.0	0.9996	1.56	2.85	99.13±3.19	2.46
Cs	0.0—100.0	0.9998	1.76	2.67	102.83±4.01	2.01
Ba	0.0—100.0	0.9999	1.74	3.69	114.36±5.23	2.50
Ce	0.0—10.0	1.0000	1.18	2.87	103.92±1.62	2.28
Hg	0.0—1.0	0.9994	3.74	4.23	92.22±2.78	6.74
Tl	0.0—1.0	0.9996	3.52	3.16	96.15±6.37	10.17
Pb	0.0—100.0	0.9999	2.12	4.36	97.26±4.18	5.95
Th	0.0—1.0	0.9996	2.05	5.32	107.71±5.20	7.23
U	0.0—1.0	0.9997	3.05	6.83	102.84±3.88	6.64

[a]R square is determination of coefficients. See methods in text for details. [b] Relative standard deviation (R.S.D.) values are used for evaluation of accuracy, precision, and stability.

Table S-4. Fold changes of elemental markers in different gout populations.

Element	Discovery		Validation		Shanghai c		Shandong c		Beijing c	
	FC [a]	FDR-*P*[b]	FC	FDR-*P*	FC	FDR-*P*	FC	FDR-*P*	FC	FDR-*P*
Li	2.4	<0.0001	2.6	0.0013	1.6	<0.0001	2.5	<0.0001	2.5	<0.0001
Al	0.8	0.0294	0.7	0.0355	0.7	0.0021	0.8	0.0305	0.8	0.0561
Ti	0.8	0.0367	0.7	0.0264	0.7	0.0009	0.7	0.0122	0.9	0.2873
Fe	0.8	0.0139	0.7	0.0426	0.7	0.0007	0.8	0.0279	0.8	0.0596
Cu	1.6	0.0002	1.6	0.0410	1.2	0.0299	1.5	0.0020	2.1	<0.0001
Se	0.8	0.0367	0.7	0.0264	0.7	0.0006	0.8	0.0769	0.8	0.0768
Sr	1.9	<0.0001	1.5	0.0458	1.4	0.0015	1.7	<0.0001	1.9	<0.0001
Ta	0.8	0.0156	0.7	0.0413	0.7	0.0006	0.8	0.0637	0.8	0.0257
Hg	1.3	0.0234	1.6	0.0402	1.6	<0.0001	1.1	0.2775	1.2	0.1040
Bi	0.7	0.0017	0.6	0.0020	0.7	0.0028	0.6	0.0018	0.6	<0.0001
Th	0.7	0.0003	0.6	0.0020	0.6	<0.0001	0.6	0.0011	0.6	0.0002
U	0.7	0.0004	0.6	0.0033	0.5	<0.0001	1.0	0.9668	0.7	0.0024

[a]Fold changes are the ratios of the gout patients versus the healthy controls using Mann-Whitney test. The value greater than 1.0 indicates a higher concentration of the element while the value lower than 1.0 suggests a lower concentration in gout patients as compared to the healthy controls. [b]False discovery rate (FDR) corrected Mann-Whitney *P* values. [c]The gout patients from these three locations included all the patients from each location.

Table S-5. Diagnostic performance evaluation of the combinations of elemental markers.

LR method [a]	Elements used	Discovery group				Validation group			
		AUC ± S.E. [b] (95% CI)	*P* [c]	Sens. [d] (95% CI)	Spec. [d] (95% CI)	AUC ± S.E. (95% CI)	*P*	Sens. (95% CI)	Spec. (95% CI)
Enter	Li*, Al*, Ti, Fe, Cu, Se, Sr, Ta, Hg, Bi, Th, U*	0.99 ± 0.004 (0.98-1.00)	<0.0001	0.97 (0.92-0.99)	0.95 (0.83-0.99)	1.00 ± 0.000 (0.00-1.00)	<0.0001	1.00 (0.91-1.00)	1.00 (0.75-1.00)
Backward	Li*, Al*, Ti, Se, Hg, Th, U*	0.99 ± 0.006 (0.98-1.00)	<0.0001	0.98 (0.93-1.00)	0.98 (0.87-1.00)	1.00 ± 0.000 (0.00-1.00)	<0.0001	1.00 (0.91-1.00)	1.00 (0.75-1.00)
Forward	Li*, Al*, U*	0.98 ± 0.011 (0.96-1.00)	<0.0001	0.98 (0.93-1.00)	0.90 (0.76-0.97)	0.98 ± 0.022 (0.94-1.00)	<0.0001	0.97 (0.87-1.00)	1.00 (0.75-1.00)

[a] Binary logistic regression (LR) with three different variable selection methods (i.e., Enter, Backward Likelihood Ratio, and Forward Likelihood Ratio); [b] The area under the Receiver operating characteristic (ROC) curves (AUC); [c] P values were calculated by ROC analysis; * These elements are statistically significant ($P < 0.05$) using LR with Wald test.

Table S-6. Comparison of AUC for different combinations of gout elemental markers.

Contrast	Discovery			Validation		
	Difference	S.E.	*P*	Difference	S.E.	*P*
Enter versus LR-Forward	0.02	0.008	0.0453	0.02	0.022	0.3209
Enter versus LR-Backward	0.00	0.002	0.4663	0.00	0.000	-
Enter versus Li	0.14	0.030	<0.0001	0.13	0.049	0.0095
Enter versus Al	0.37	0.050	<0.0001	0.28	0.070	<0.0001
Enter versus U	0.28	0.044	<0.0001	0.17	0.057	0.0022
LR-Forward versus LR-Backward	-0.01	0.007	0.0532	-0.02	0.022	0.3209
LR-Forward versus Li	0.12	0.029	<0.0001	0.10	0.043	0.0154
LR-Forward versus Al	0.35	0.050	<0.0001	0.25	0.074	0.0006
LR-Forward versus U	0.26	0.043	<0.0001	0.15	0.060	0.0115
LR-Backward versus Li	0.14	0.029	<0.0001	0.13	0.049	0.0095
LR-Backward versus Al	0.37	0.051	<0.0001	0.28	0.070	<0.0001
LR-Backward versus U	0.27	0.044	<0.0001	0.17	0.057	0.0022
Li versus Al	0.23	0.069	0.0008	0.15	0.094	0.1123
Li versus U	0.14	0.063	0.0302	0.05	0.085	0.5768
Al versus U	-0.09	0.037	0.0111	-0.10	0.055	0.0623

H0: Difference between areas = 0. H1: Difference between areas ≠ 0.$P<$ 0.05 indicates that there is a significant difference of AUC between two given combinations or elements.

Table S-7. Age- and urate- adjusted *P* values for elemental markers using LR.

Element	*P* a
Li	0.0010
Bi b	0.9293
Th	0.0023
U	0.0001
Ti	0.0101
Se	0.0061
Al	0.0152
Hg	0.0364
Cu	0.0087
Ta	0.0041
Fe	0.0100
Sr	0.0098

[a]P values were attained by LR with Wald test and the critical value is 0.05 in this study. [b] Element is not significantly changed after age- and urate-adjustment.

Table S-8. Differential elements between two different gout stages.

Element	FC [a]	FDR-*P* [b]
Cd	0.7	0.0347
Tl	0.7	0.0363
Cs	0.7	0.0363
Rb	0.7	0.0363
Hg	0.7	0.0376
Ag	1.3	0.0447

[a]Fold changes are the ratios of the patients at acute stage versus those at interval stage using Mann-Whitney test. A value greater than 1.0 indicates a higher concentration of the element while a value lower than 1.0 suggests a lower concentration in the patients at acute stage as compared to those at interval stage. The patients (n = 65) were from Shanghai city because of the patient's stage information was available in this population. [b] False discovery rate (FDR) corrected Mann-Whitney *P* values.

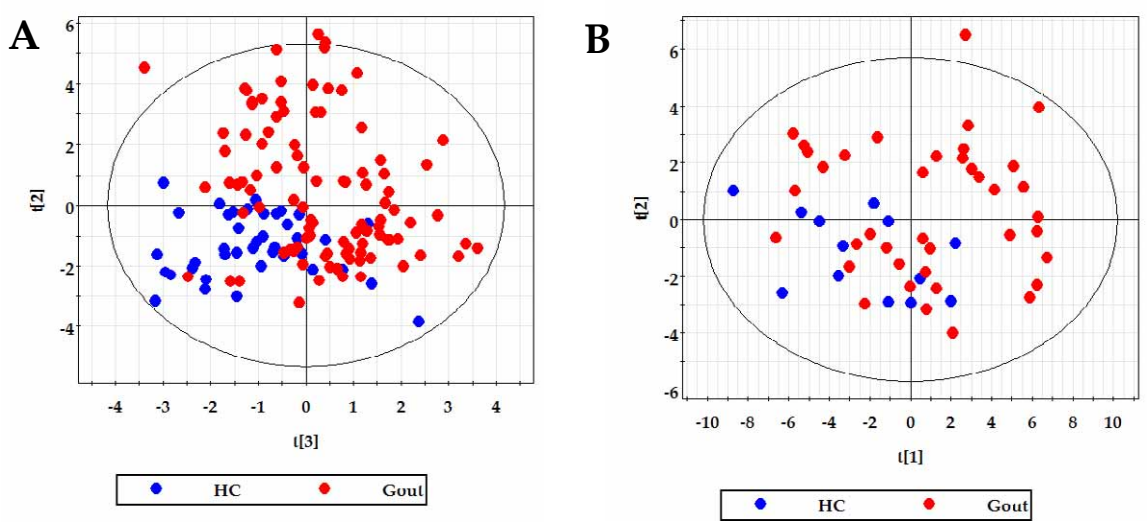

Figure S-1. PCA scores plot showing separation trend between the gout patients versus the healthy controls from the discovery group (A, $R^2X_{cum(10PCs)}$=0.61) and from the validation group (B, $R^2X_{cum(8PCs)}$=0.61). Each dot in the plot represents an individual subject from the healthy controls (HC) (blue, n = 40 for the discovery group or n = 13 for the validation group); and from gout patients (Gout, red, n = 100 for the discovery group or n = 39 for the validation group).

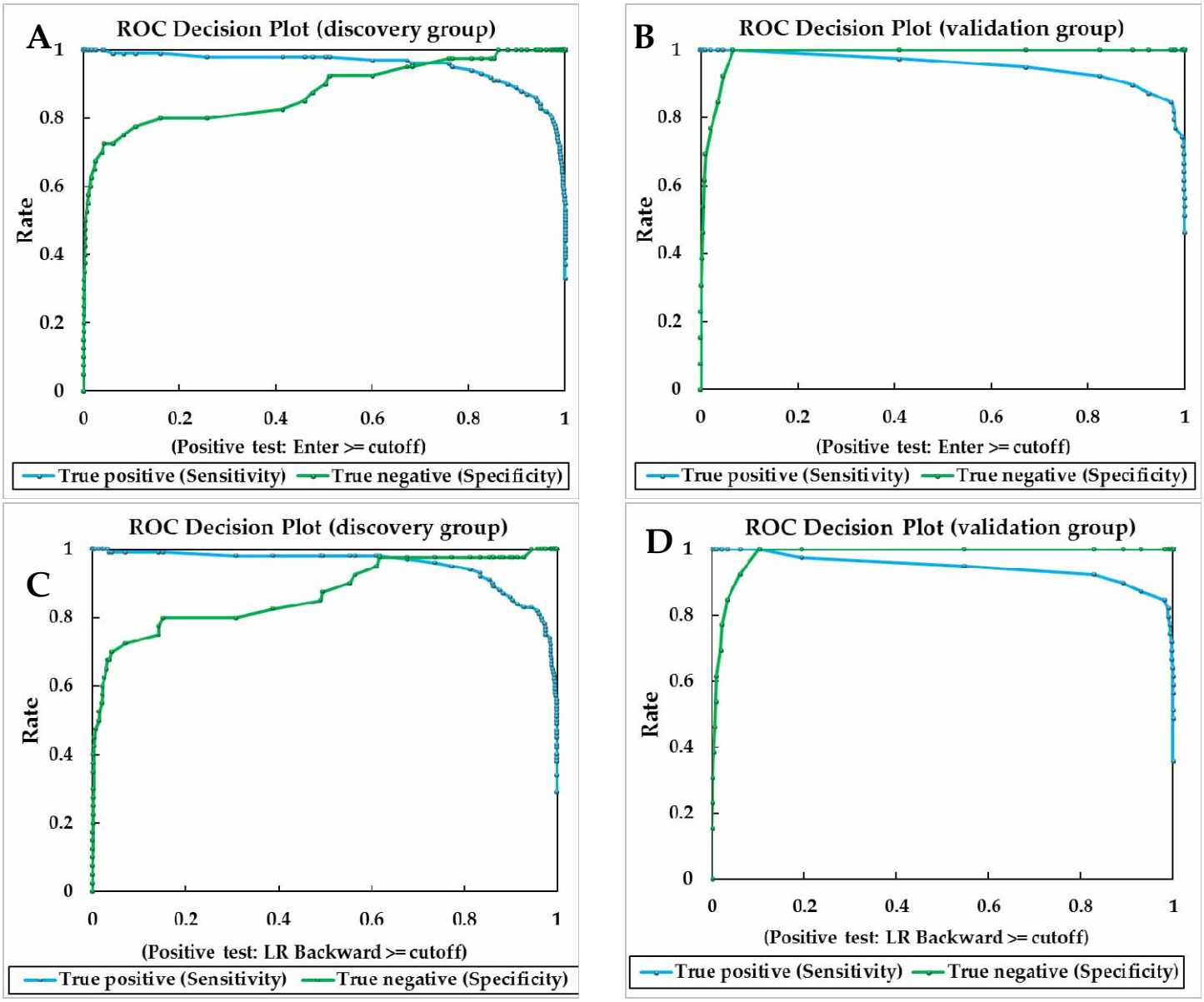

Figure S-2. ROC decision plots showing an optimum decision threshold where the maximum number of cases is correctly diagnosed as gout patients/healthy controls in the discovery group (A, C) and the validation group (B, D) using LR with Enter and Backward Likelihood Ratio.

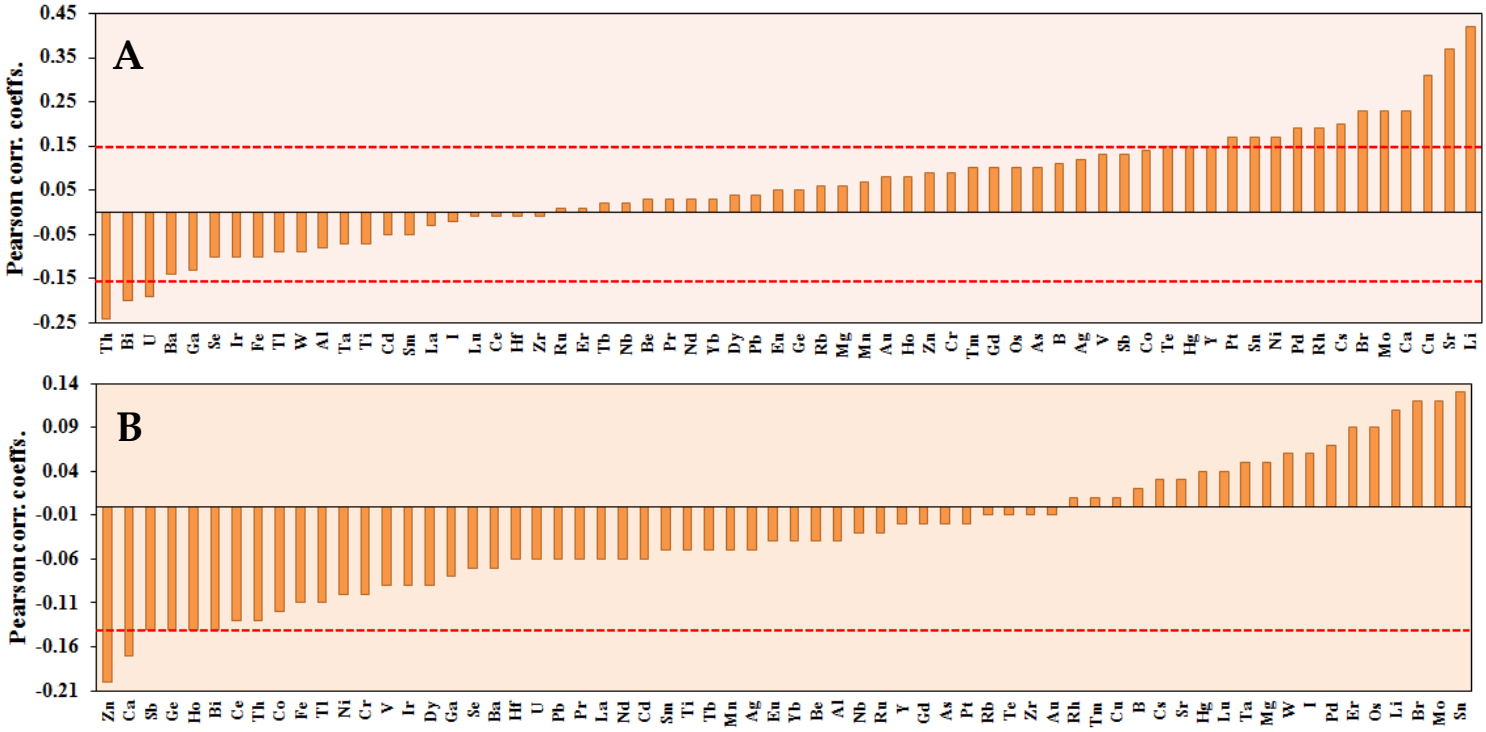

Figure S-3. Pearson's correlation between each individual element and blood uric acid (A) and between each element and age (B) in all the subjects (n = 192). The significant correlation is defined as "|r|≥0.15, *P*<0.05" (red dash line) andeach bar represents each individual element. The positive value suggests a positive correlation whereas the negative values are inverse correlation. The concentrations of elements and serum urate are logarithm (base 10) transformed.

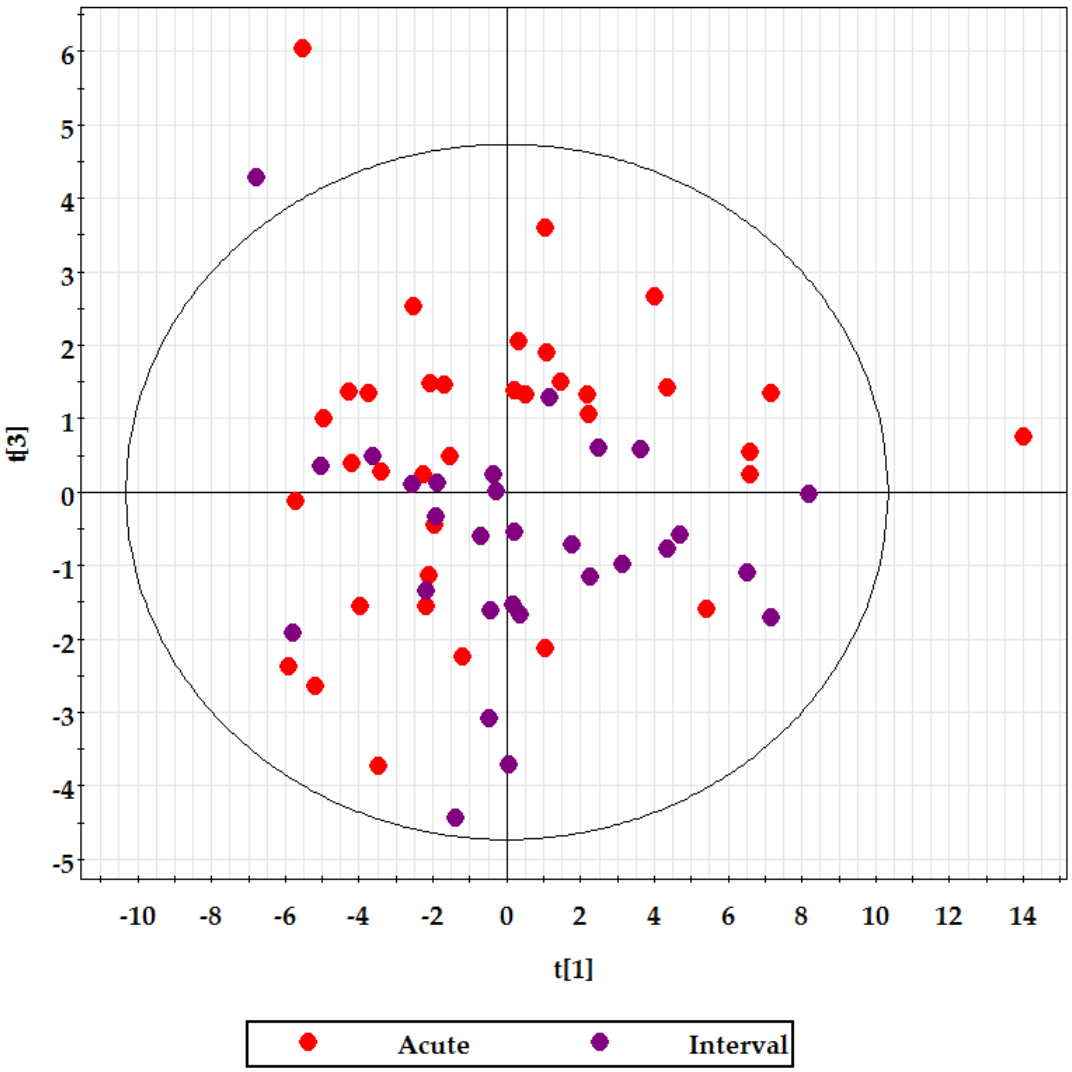

Figure S-4. PCA scores plot (9 components, $R^2X_{cum(9PCs)}$=0.61) showing a slight separation trend between the two gout stages i.e., acute versus interval stages. The gout patients (n = 65) used were from Shanghai city and each dot in the plot represents an individual subject from gout patients at acute stage (red, n = 36) and from patients at interval stage (violet, n = 29).